NOTE ON CERTAIN VARIATIONS OF THE SPOROCYST IN A SPECIES OF *SAPROLEGNIA*.*

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(With eleven Text-figures.)

Introduction.

During the summer of 1919, a quantity of *Saprolegnia* was made available to the writer by the accidental drowning of a beetle, in a tank situated in the glass-house of the Botany Department, University of Adelaide. The material, with a little water from the tank, was transferred to the laboratory and kept under observation for some weeks. It was found to show variations in sporocyst formation similar to those recorded by Lechmere† for *Saprolegnia Thureti* and certain additional variations which are thought to be of sufficient interest to place on record.

Since sexual reproduction was not observed, it was impossible to identify the species under examination with any accuracy. Judging by the stout nature of the hyphae, however, it seems probable that the species is identical with that of Lechmere's work, *Saprolegnia Thureti*.

The writer's thanks are due to Professor T. G. B. Osborn, University of Adelaide, in whose Department these observations were made, for his interest in the work.

Previous Investigations.

In his investigation of certain species of *Saprolegnia* Lechmere‡ draws attention to the marked variability in the nature of sporocyst formation and discharge. In addition to the normal *Saprolegnia* type of sporocyst, he records five variations, each of which shows features which are characteristic of a distinct genus of the *Saprolegniaceae*. These variations are all concerned with the nature of discharge of the sporocysts, and sometimes result in the suppression of the first, second or of both motile phases. The following is a brief summary of the sporocyst variations observed by Lechmere:—

1. *Leptolegnia* condition, where the zoospores are arranged in a single row in a long cylindrical sporocyst. Two motile phases.

2. *Pythiopsis* condition, where the spores swarm feebly from a club-shaped sporocyst, and come to rest near the mouth of the sporocyst. No record of a second motile phase.

*The observations recorded in this paper were made while the writer held the position of Demonstrator in Botany, The University of Adelaide.

†New Phytologist, ix., 1910, p.308.

‡*Loc. cit.*

3. *Achlya* condition, where the first motile phase is merely represented by the liberation of the spores, which encyst in a mass near the mouth of the sporocyst. The second motile phase occurs later.

4. *Dictyuchus* condition, where the spores encyst within the sporocyst, regardless of the terminal opening formed for their exit. Later, the spores leave their cyst cases, enter upon the second motile phase, pass out through the terminal opening, often pushing their empty cyst cases before them. A second *Dictyuchus* condition is described* where the zoospores pass directly through the wall of the sporocyst. There is no definite statement as to how the zoospores pass through the sporocyst wall, but from Lechmere's figure 17 we can only infer that the sporocyst wall degenerates at certain places to allow the escape of the zoospores.

5. *Aplanes* condition, where all motility is suppressed, and germination is direct within the sporocyst, the germ tubes growing out through the wall of the sporocyst.

A variation of the sporocyst known as the "*Dictyuchus*-form" was recorded for the *Saprolegniaceae* prior to Lechmere's work. Hartog† refers to this form in his analysis of the genera in the *Saprolegniaceae*, as follows:—

"*Dictyuchus*-form. When the spores of *Achlya* or *Saprolegnia* fail to leave it [the sporocyst] at maturity, they encyst within, constituting this form or dictyosporange. They either swarm ultimately in the second form or germinate in situ by emission of a hypha." The "*Dictyuchus*-form" of Hartog is evidently the dictyosporocyst of Lechmere which includes the *Dictyuchus* and *Aplanes* conditions.

In the present investigation, which was made upon material growing under natural conditions as well as upon white of egg cultures, the writer observed sporocysts of the *Leptolegnia*, *Pythiopsis*, *Achlya* and *Aplanes* types similar to those described by Lechmere. In addition, certain remarkable composite sporocysts were observed which combine the characters of from two to four genera; also a new *Dictyuchus* condition which differs from either of those described by Lechmere.‡

Material was selected from the body, legs, wings and antennae of the insect in order to ascertain whether the sporocyst variations were restricted to any particular part of the host, and whether their occurrence is influenced by the amount of available nutriment. Although the general growth of the hyphae was found to be more vigorous upon the body of the insect, the abnormal sporocysts occurred freely on all parts.

Upon transferring part of the material to tap water in the laboratory, the production of sporocysts was stimulated in both old and young hyphae. Young hyphae show a preponderance of the normal *Saprolegnia* type of sporocyst in which two motile phases were observed. In old hyphae, however, the new sporocysts often form within or at the base of three or four discharged sporocysts. In these cases some of the new zoospores find difficulty of escape, and encyst during their passage through the old discharged sporocysts (Text-fig. 1). Encystment within the sporocyst under these conditions is found to occur at approximately the same time as that of zoospores which succeed in escaping. Retained zoospores

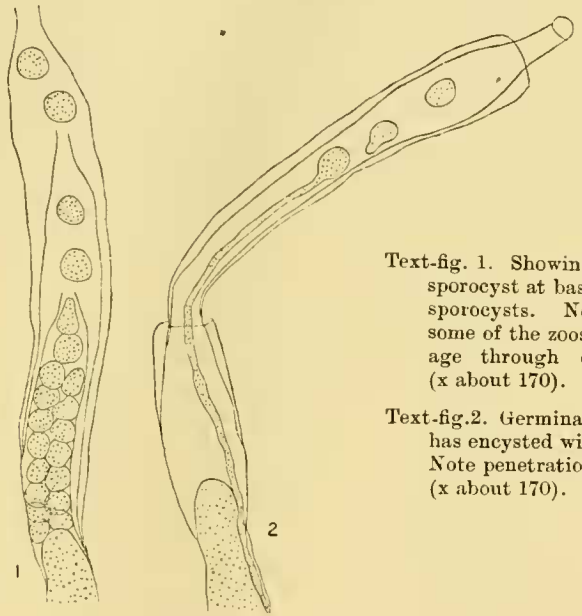
*New Phytologist, x., 1911, pp.167-203.

†Ann. Bot., xi., 1888-89, p.203.

‡New Phytologist, ix., 1910, p.308; and x., 1911, p.167.

which encyst in the above manner, do not enter upon a second motile phase, but germinate directly within the sporocyst. In some cases a germ tube from an encysted zoospore has been observed to grow back through a series of empty sporocysts and penetrate the hypha behind for some distance (Text-fig. 2). The *Aplanes* type of germination by penetration of the wall of the sporocyst, does not occur under these circumstances.

In cultures made upon white of egg, early sporocyst formation was decidedly of the *Saprolegnia* type, but after some days, all the variations recorded appeared in the culture. Owing to the rapidity of their formation, it was impos-



Text-fig. 1. Showing formation of new sporocyst at base of old discharged sporocysts. Note encystment of some of the zoospores during passage through empty sporocysts. (x about 170).

Text-fig.2. Germination of spore which has encysted within old sporocyst. Note penetration of hypha behind. (x about 170).

sible to discover any definite sequence of formation of sporocyst types. It was found, however, that normal sporocysts occur more abundantly in the young culture, though they continue to be formed to a slight degree, after the abnormal forms have made their appearance. *Leptolegnia, Pythiopsis* and *Achlya* conditions were found to be more rare than *Dictyuchus* and *Aplanes* conditions.

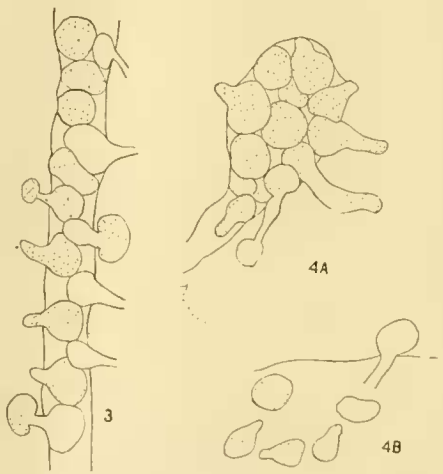
The most noticeable feature of the material was the prevalence, under both natural and cultural conditions, of two forms of sporocyst unrecorded by previous investigators. The first of these is certainly a *Dictyuchus* condition, though it differs in salient features from either of the *Dictyuchus* conditions described by Lechmere. In reference to the formation of the *Dictyuchus* condition on a white of egg culture, Lechmere says:—

"The numerous resting sporocysts present on the mycelium commenced to develop into sporocysts. In by far the greater number of cases observed, the spores encysted within the sporocyst before its discharge, in spite of the fact that a tubular process was developed on the sporocyst before the spores encysted."*

*New Phytologist, ix., 1910, p.316, Pl. 2, figs. 30, 31.

Also, "The zoospores were observed leaving their cyst walls and escaping through the tubular process, pushing before them the empty cyst cases which were in the tube."† Lechmere only observed this condition during the development of resting sporocysts in white of egg cultures. It is interesting to note that here the second motile phase commences within the sporocyst. A further *Dictyuchus* condition is recorded by Lechmere in his second paper.‡ He says of these sporocysts: "they are always derived from gemmae, and in shape they are broad and short. The empty spore cases form a dense network within the sporocyst, the wall of which is very thin and is apparently directly penetrated by the zoospores on their escape from their encysted condition."

The *Dictyuchus* condition observed by the writer, resembles the second of Lechmere's *Dictyuchus* forms in the fact that the second motile phase occurs outside the sporocyst, the first being suppressed. The sporocysts are found to be either short and club-shaped, or long and cylindrical; they are never observed to arise from resting sporocysts and occur freely under natural as well as cultural conditions. The encysted spores send out a protuberance which penetrates the sporocyst wall and projects for a distance of varying length (Text-fig. 3). The protoplast then shrinks from the cyst wall, streams out through the cyst tube, the



Text-fig.3. Portion of young *Dictyuchus* sporocyst. Note germination of encysted spores and passing of protoplast through cyst tube. (x 230).

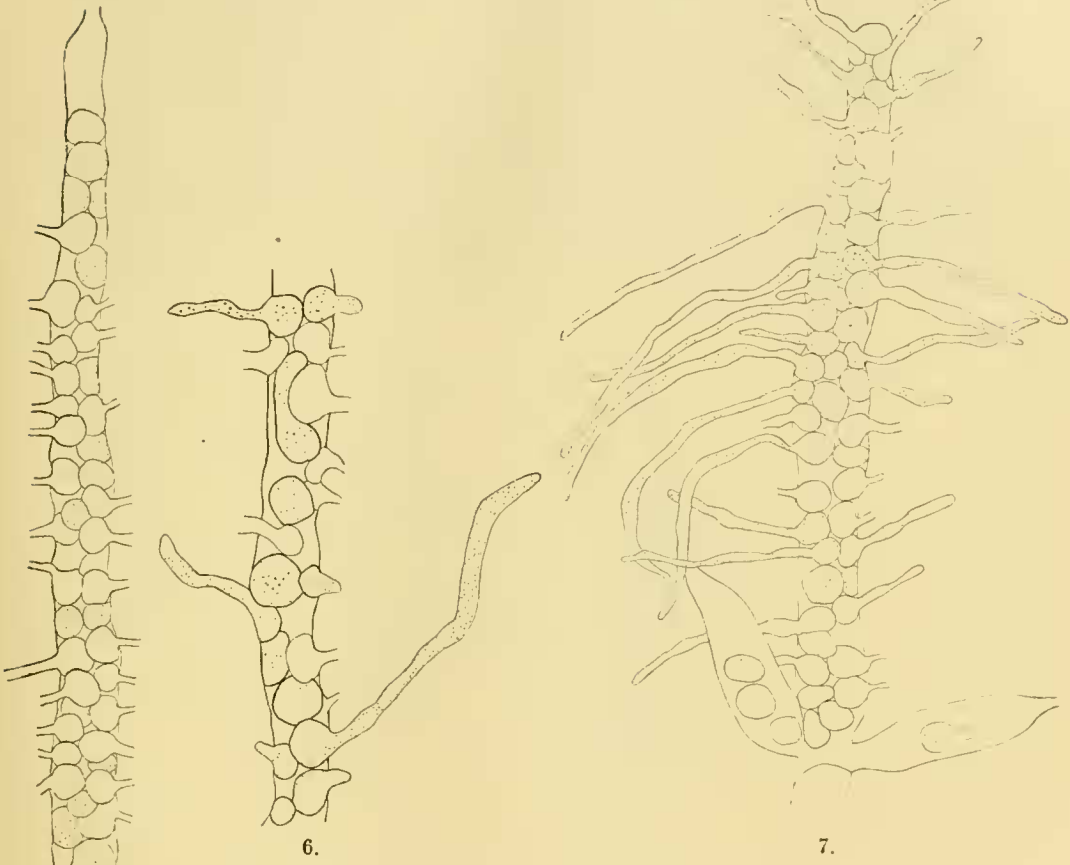
Text-figs.4*b*. & 4*a*. Club-shaped *Dictyuchus* sporocyst showing liberation of ciliated zoospore and the coming to rest and second encystment of the latter. (x 230).

tip of which degenerates. A ciliated zoospore is liberated which remains attached to the cyst tube for about five minutes, maintaining a rocking motion. It then swims away and comes to rest close to the sporocyst after a period of from 3—5 minutes (Text-figs. 4*b* and 4*a*). This motility is noticeably feeble in comparison with the vigorous motility in Lechmere's first *Dictyuchus* condition where the zoospores push their empty cyst cases before them when leaving the sporo-

†*Id.*, ix., 1910, p.317.

‡*Id.*, x., 1911, p.186, fig. 17.

cyst. Direct germination follows this second encystment. This mode of discharge, through cyst-tubes which penetrate the wall of the sporocyst, is similar to the mode of discharge described and figured by Lotsy for the genus *Dictyuchus*.§



5. 6. 7.

Text-fig.5. *Dictyuchus* type of sporocyst incompletely discharged. Note projecting cyst tubes from empty cyst cases. (x about 170).

Text-fig.6. Portion of a cylindrical sporocyst showing combination of *Dictyuchus* and *Aplanes* conditions. (x 230).

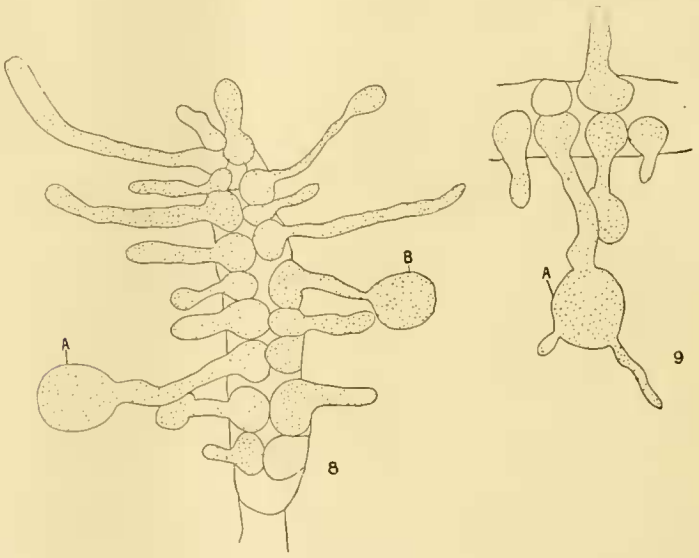
Text-fig.7. Composite sporocyst showing predominance of "*Dictyu-Aplanes*" condition. Note *Achlya* type of branching with lateral sporocysts of normal *Saprolegnia* form. In the latter, complete discharge has led to encystment and germination within the sporocyst. (x about 170).

The partly or wholly discharged sporocysts with their empty cyst cases and projecting cyst tubes with abruptly broken tips, present a peculiar and striking appearance (Text-fig. 5). In some instances there is evidence of a primary attempt at discharge of the sporocyst in the normal *Saprolegnia* manner through a terminal opening (Text-fig. 5).

§Lotsy, Pflanzensystematik, i., 1907, p.164, fig. 95,

In the opinion of the writer the above is the true *Dictyuchus* condition, that described by Lechmere being a transitional stage to this condition.

In addition to a true *Dictyuchus* and a true *Aplanes* condition, an interesting transition often occurs which combines the features of both these types. In certain sporocysts a number of encysted spores germinate directly, producing long narrow tubes resembling young hyphae, which penetrate the sporocyst wall. Other encysted spores, within the same sporocyst, after producing a cyst tube, cease to germinate and enter upon a *Dictyuchus* condition, the second motile phase taking place with the liberation of a zoospore from a broken cyst tube (Text-fig. 6). This type of sporocyst is a combination of that where all motility is suppressed (*Aplanes*) with one in which the second motile phase is present, although noticeably feeble and short in duration. It is interesting to note that transitional forms occur, between the true *Dictyuchus* condition, through composite sporocysts where either the *Dictyuchus* or *Aplanes* condition is predominant, to the pure *Aplanes* form. It is suggested that this composite sporocyst should be known as the "*Dictyu-Aplanes*" condition.



Text-fig.8. Club-shaped sporocyst of the *Aplanes* form. Swollen structures at "a" and "b" are formed by the streaming of the protoplasm towards the tip of the germ tube. (x 230).

Text-fig.9. Shows formation of two germ tubes in terminal swelling after streaming of protoplasm has ceased. (x 230).

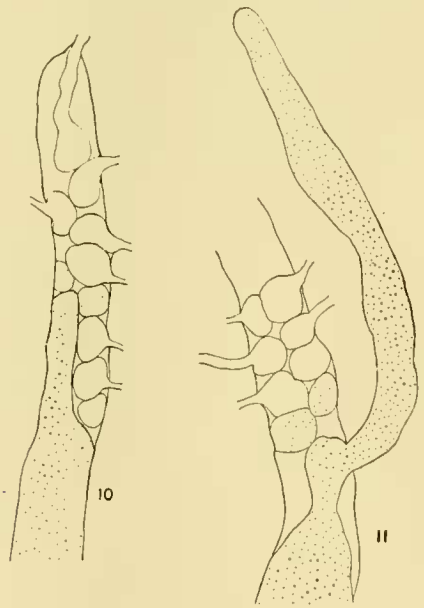
Rare examples of composite sporocysts are found, in which the features of *Achlya, Saprolegnia, Dictyuchus* and *Aplanes* are combined. In Text-fig. 7 the *Achlya* type of branching is shown. The main sporocyst is of the "*Dictyu-Aplanes*" form, while the two basal sporocysts are normal *Saprolegnia* sporocysts incompletely discharged.

An interesting feature often occurs in the *Aplanes* type of sporocyst, in what may be regarded as an attempt at escape of the protoplast during germination. After some of the germ-tubes have reached a certain length, a streaming movement is observed from the body of the germinating spore. Part of the protoplasm streams to the tip of the tube, where a large swollen structure is formed (Text-fig. 8). This swollen structure never ruptures the tip of the germ-tube, but appears to function as an encysted spore from which one or two germ-tubes may ultimately develop (Text-fig. 9).

From these observations it seems conceivable that the *Aplanes* condition has arisen from the *Dictyuchus* condition by the delay of protoplasmic activity until the length of the germ-tube renders escape ineffective.

Formation of New Sporocysts.

Owing to the prevalence of *Dictyuchus*, *Aplanes*, and "*Dictyu-Aplanes*" forms in which the original sporocyst remains blocked either by empty cyst cases or by



Text-fig. 10. Short *Dictyuchus* sporocyst showing penetration of hypha at base in attempt to form new sporocyst. (x 230).

Text-fig. 11. Base of old *Dictyuchus* sporocyst showing lateral divergence of hypha to form new sporocyst. (x 230).

germinating spores, the formation of a new sporocyst almost invariably takes place by the lateral outgrowth of the hypha from the base of the sporocyst. The hypha grows forward until further progress is blocked, lateral divergences then taking place (Text-figs. 10 and 11). In this manner a pseudo-*Achlya* condition is obtained.

Summary.

1. Certain species of *Saprolegnia* are known to show variations in sporocyst formation and discharge, when grown under cultural conditions.
2. In the present investigation certain variations are recorded for an undetermined species of *Saprolegnia* growing under natural as well as cultural conditions. *Leptolegnia, Pythiopsis* and *Achlya* conditions occurred rarely, while *Dictyuchus* and *Aplanes* conditions were found frequently. These variations occurred in both club-shaped and cylindrical sporocysts, but were not observed arising from resting sporocysts.
3. The *Dictyuchus* condition described here differs from either of those described by Lechmere and is held to be the true *Dictyuchus* condition.
4. Composite sporocysts were observed, the most important of which combine the features of *Dictyuchus* and *Aplanes*. The name "*Dictyu-Aplanes*" is suggested for these sporocysts.
5. Evidence is given in favour of the suggestion that the *Aplanes* condition has arisen from the *Dictyuchus* condition, by failure of the protoplast to escape from the germ-tube during its early growth.
6. New sporocysts are frequently formed as lateral, basal branches of old sporocysts, owing to the blocking of the latter with empty cyst cases and germinating spores.

All Text-figures were made at table level, with Zeiss camera lucida and tube at 160 mm., Leitz objectives 3 and 6 and oculars 2 and 4.